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Selective isolation of β -glucan from corn pericarp hemicelluloses by affinity chromatography on cellulose column.

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1 **Selective Isolation of β -Glucan from Corn Pericarp Hemicelluloses**

2 **by Affinity Chromatography on Cellulose Column**

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Abstract

A combination of anion-exchange chromatography and affinity chromatography on a cellulose column was found to be effective for the isolation of β -(1,3;1,4)-glucan (BG) from corn pericarp hemicelluloses (CPHs). CPHs containing 6.6% BG were extracted from corn pericarp with 6 M urea-2 wt% NaOH solution and initially fractionated into neutral and acidic parts by anion exchange chromatography to remove acidic arabinoxylan consisting of arabinose (35.6%) and xylose (50.9%). The neutral fraction (yield; 10.1% on the basis of CPHs) consisting of 1.0% arabinose, 10.1% xylose and 80.3% glucose containing 28.4% BG was then applied to a cellulose column of Watman CF-11. BG could be recovered from the adsorbed fraction on the cellulose column by elution with 2% NaOH in a yield of 2.6% on the basis of CPHs with a purity of 84.7%. The chemical structure of the isolated corn pericarp BG was confirmed by ^{13}C -NMR spectroscopic, methylation and lichenase treatment analyses. The results indicate that the ratios of (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segments of the BG were 2.60 and 2.5, respectively.

Keywords: β -Glucan; Corn pericarp hemicelluloses; Cellulose column; Affinity chromatography

1. Introduction

Corn pericarp is a by-product of industrial corn starch production and its annual global generation is estimated to be over 4 million tons (Yoshida, Dwianto, Honda, Uyama, & Azuma, 2014). Although it is frequently used as an ingredient in animal feed with the addition of corn protein (Shukla, & Cheryan, 2001), finding out more valuable applications is expected. Because hemicelluloses are its major constituents amounting to about 75%, its functional use largely depends on their extended characterization. Use of corn pericarp hemicelluloses (CPHs) as an emulsifier is a candidate for this line of investigation (Yadav, Johnston, & Hicks, 2007; Yadav, Parris, Johnston, & Hicks, 2008).

Recently we found that corn pericarp contains 3.2% of β -(1,3;1,4)-glucan (BG) (Yoshida, Sakamoto, & Azuma, 2012). BG is commonly present in cereal grains and is included in many kinds of commercially available cereal based foods as a nutritionally important ingredient, because it improves food qualities such as mouthfeel and texture (Lazaridou, & Biliaderis, 2007). In addition, BG provides some specific health benefits, such as attenuating blood postprandial glycemic and insulinemic responses, lowering blood total cholesterol and low-density lipoprotein (LDL) cholesterol, and improving high-density lipoprotein (HDL) cholesterol and blood lipid profiles (Braaten *et al.*, 1994; Daou, & Zhang, 2012; Brennan, & Cleary, 2005).

BG is a linear homopolysaccharide comprised of two types of D-glucopyranosyl residues linked by a mixture of β -(1-3) and β -(1-4) linkages, with blocks of (1-4)-linked residues (oligomeric cellulose-like segments) separated by (1-3)-linkages. Its structural

features, such as linkage ratio, number of units of cellulose-like segments and distribution of the cellulose-like segments, are known to be important determinants for its physical properties and functionalities, including its use as a food additive (Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004).

Previously we demonstrated the effectiveness of a NaOH-urea solvent system for extraction of hemicelluloses from corn pericarp, including BG (Yoshida, Sakamoto, & Azuma, 2012). Although biorefinement of corn pericarp targeted to produce BG is desirable as it is an innovative utilization of corn starch residues, rather tedious steps for the removal of large amounts of other polysaccharides are usually required for the isolation of BG from monocotyledonous crops (Ahmad, Anjum, Zahoor, Nawaz, & Ahmed, 2009; Ahmad, Anjum, Zahoor, Nawaz, & Din, 2007; Beer, Arrigoin, & Amadò, 1996; Bhatt, 1993; Burkus, & Temelli, 1998; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Wood, Weisz, Fedec, & Burrows, 1989). Two important steps so far noticed were removal of contaminating starch and arabinoxylan. Repeated treatments with thermo-stable starch-degrading enzymes were usually necessary to enrich BG for removal of starch. Solubility difference in aqueous media was frequently used to remove arabinoxylan (Izydorczyk, & Biliaderis, 1995; Izydorczyk, Biliaderis, Macri, & MacGregor, 1997; Izydorczyk, & MacGregor, 2000). However, in the case of CPHs, removal of starch was not prerequisite because of the low content (about 1%) and our trials of fractional precipitation of BG from a mixture with arabinoxylan by using ethanol and ammonium sulfate were unsuccessful.

In this study, we developed a convenient isolation method specific for corn pericarp

BG by using affinity chromatography on a cellulose column, and present the chemical properties of the isolated BG were also investigated.

2. Materials and Methods

2. 1. Materials

Kernels of sweet corn cultivated and steamed for food in Hokkaido, Japan, were purchased from Kewpie Co., Japan. Corn pericarp was manually peeled from the upper portion of each kernel ($9.4 \pm 0.7\%$ on basis of dried kernel, $n = 10$) and treated with hot water (121°C) for 1 h. Corn pericarp hemicelluloses (CPHs) consisting of 40.5% of xylose, 29.2% of arabinose, 26.2% of glucose and 4.1% of galactose were prepared by extraction of corn pericarp with 2 wt% NaOH-6 M urea in a yield of 74.8% on the basis of dried corn pericarp as described previously (Yoshida, Sakamoto, & Azuma, 2012). β -Glucan (BG) from barley ($>95\%$) was purchased from Sigma (St. Louis, Missouri, USA). Amounts of BG and starch were determined by using the mixed-linkage β -glucan and total starch content assay kits (Megazyme International Ireland Ltd., Wicklow, Ireland), respectively. Whatman CF-11 cellulose powder (WhatmanTM, a part of GE Healthcare Life Science, Ltd., Buckinghamshire, UK) was used for affinity chromatography after pre-washing with 5% NaOH and neutralization with acetic acid.

2. 2. Isolation of β -(1,3;1,4)-glucan

Anion exchange chromatography was first applied for partial purification of BG. Hot water-soluble CPHs ($94.2 \pm 0.5\%$ on the basis of CPHs, $n = 4$) obtained by extraction of

corn pericarp with a 100 fold excess of water at 80°C for 2-3 h were applied to a column (15 × 150 mm) of TOYOPEARL DEAE-650M (Tosoh Co., Tokyo, Japan) equilibrated with 5 mM sodium phosphate buffer (pH 6.8) and eluted with the same solution to recover neutral BG. Acidic arabinoxylan was next recovered by elution with the same buffer containing 1.2 M NaCl. Elution was monitored by the phenol-sulfuric acid method. Both polysaccharide fractions were separately pooled, dialyzed against water and freeze-dried. The BG-rich fraction (Neutral fraction; $8.7 \pm 2.4\%$ on the basis of CPHs, $n = 3$, Table 1) was dissolved in 5 mM sodium acetate buffer (SAB), pH 5.0 and applied to a cellulose column (15 × 150 mm). After equilibrating for 30 min at room temperature, the column was washed with the same buffer to remove unadsorbed material, eluted with distilled water, and finally adsorbed BG was recovered by elution with 2% NaOH (2% NaOH fraction; yield $3.3 \pm 1.3\%$ on the basis of CPHs, $n = 3$, Table 1). All carbohydrate containing fractions were pooled, neutralized, dialyzed against water and freeze-dried.

2. 3. Chemical analysis

CPHs and all of the materials recovered by anion exchange and affinity chromatographic techniques were hydrolyzed according to the Saeman method (Saeman, Bubl, & Harris, 1945), and their monosaccharide compositions were determined by high-performance anion exchange chromatography (HPAEC) on a Dionex DX-500 system (Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (ED-40) as described in our previous report (Yoshida, Tusbaki, Teramoto, & Azuma, 2010).

The liquid state ^{13}C -NMR spectrum of the isolated BG was recorded in D_2O on a Bruker DPX-400 instrument (Billerica, Bruker, MA, USA) operating at 400 MHz and the chemical shifts in ppm were normalized as downfield values from that of internal standard, TSP (sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate).

Permethylation of polysaccharides was carried out according to the Hakomori method (Hakomori, 1964). The permethylated polysaccharides were subjected to two-step hydrolysis with 90% formic acid for 2 h at 100°C and 0.5 N sulfuric acid for 12 h at 100°C . After neutralization with barium carbonate, the hydrolyzate was reduced with sodium borohydride and acetylated with a mixture of acetic anhydride and pyridine (1:1, v/v) for 1 h at 100°C . The resulting mixture of partially methylated alditol acetates was analyzed by GC/MS with a Shimadzu Parvum 2 (70 eV) using a column of Shimadzu CBP-1 (0.25 μm , 0.25 mm \times 25 m) and a linear temperature gradient from 140°C to 220°C at $2^\circ\text{C}/\text{min}$.

The distribution of (1,4)-linked-glucopyranosyl segments in BG was determined by lichenase treatment and high-performance liquid chromatography (HPLC). BG samples were dissolved in sodium phosphate buffer (20 mM, pH6.5) and incubated with lichenase [(1,3;1,4)-beta-glucan-4-glucanohydrolase, 1000 U/mL, included in the Megazyme kit for measurement of BG content] for 2 h at 50°C . After centrifugation, the supernatant was purified by passage through a joint column of cation (Dowex 50x, 8 H^+ form) and anion (Dowex 1x8, acetate form) exchange resins. The passed solution and washed solution with pure water were freeze-dried (recovery, 62.7%). The distribution of segments was analyzed by HPLC on a column of MCI GEL CK04SS (7.5 \times 200 mm,

144 Mitsubishi Chemical Industry Co., Tokyo, Japan) at 80°C with refractive index detector
145 (RI-8, Tosoh Co., Tokyo, Japan). The eluent was deionized water and flow rate was 0.3
146 mL/min. Elution was monitored using Chrom NAV Station, Jasco, Co., Tokyo, Japan).
147

3. Results and discussion

CPHs (BG content 6.6%) were separated into neutral and acidic fractions by anion exchange chromatography on a DEAE-column as shown in Fig. 1 (A). The neutral fraction was further separated into three fractions by affinity chromatography on a cellulose column as shown Fig. 1 (B). Yields of the separated fractions are listed in Table 1. The relative monosaccharide compositions of CPHs and the fractions separated by anion exchange and affinity chromatography are listed in Table 1. The results indicate that the neutral fraction contained BG (28.4%) together with a small amount of xylan (10.1%). On the other hand, the acidic fraction was predominantly arabinoxylan consisting of xylose (50.9%) and arabinose (35.6%), with glucose as a minor constituent (3.5%). These results indicate that anion exchange chromatography was effective for partial purification of BG in corn pericarp. Previously, Gruppen *et al.* (1992) have reported that anion exchange chromatography is an efficient tool for the fractionation of arabinoxylans present in wheat flour. On the other hand, our results indicate its suitability for the removal of the acidic arabinoxylan present in abundance in CPHs.

In the present study, affinity chromatography on a cellulose column was found to be more effective for selective purification of BG. The glucose contents of the fractions eluted with SAB, distilled water and 2% NaOH were 29.5, 50.6 and 91.5%, respectively (Table 1). The starch content of the SAB, water and 2% NaOH fractions were 4.3, 5.7 and 3.5%, respectively. BG was recovered in 84.7% purity from the column by elution with 2% NaOH (Table 1) on the basis of hot water (100°C)-soluble materials. When the BG content in the NaOH fraction was initially examined by using its whole amount, BG

content was calculated as $67.0 \pm 3.6\%$. This value, however, seemed to be invalid, because this fraction was mainly consisted of glucose (91.5%) with minor contaminants of starch (3.5%) and contained a large amount of insolubilized materials (20.8%) mainly composed of glucose (78.6%). Insolubilization of BG after purification was pointed out by Lazaridou and Biliaderis (2007). Therefore, in the present study, the purity of the NaOH fraction was estimated by calculation on the basis of soluble materials.

When the water-soluble portion of CPHs was directly applied to the cellulose column, the adsorbed fraction was found to be contaminated with a larger amount of xylose (23.2%). This result shows the necessity of anion-exchange chromatography prior to cellulose affinity chromatography.

Fig. 2 shows the ^{13}C -NMR spectrum of the materials separated into the 2% NaOH fraction. Each signal was assigned according to the previous report (Bock, Duus, Norman, & Pedersen, 1991; Cui, Wood, Blackwell, & Nikiforuk, 2000; Roubroeks, Andersson, & Aman, 2000) and the peak assignments are listed in Table 2. The spectrum was identical to that of pure β -glucan (Cui, Wood, Blackwell, & Nikiforuk, 2000; Roubroeks, Andersson, & Aman, 2000). The spectrum of materials isolated in the 2% NaOH fraction showed characteristic intense peaks at 81.3 and 86.6 ppm, assigned to the signals of C-3 of (1,4)-linked- and (1,3)-linked-D-glucopyranosyl residues of β -glucan, respectively. Although very weak signals assignable to arabinoxylan (Roubroeks, Andersson, & Aman, 2000) were detected, no intense peaks other than BG could be detected. These results also indicate that the material in the 2% NaOH fraction

was high purity BG. The present study shows for the first time that BG can be effectively isolated from other hemicellulosic polysaccharides present in corn pericarp by using a combination of anion exchange and cellulose affinity column chromatography.

The glucosidic linkage analysis of the purified BG was investigated by methylation analysis. The corn pericarp BG consisted of 2,3,6-Me-Glcp, 2,4,6-Me-Glcp and 2,3,4,6-Me-Glcp in a molar ratio of 58.1 (1,4-linked Glcp), 22.3 (1,3-linked Glcp) and 0.5% (terminal Glcp), respectively (Table 3). The ratio of (1,4)-glucose linkages to (1,3)-linkages for the corn pericarp β -glucan was calculated as 2.60, which was slightly higher than that for barley (2.37) but within the ranges previously reported; 2.3-2.8 for oat, 1.9-2.8 for barley and 2.3 for rye (Lazaridou, & Biliaderis, 2007). The high affinity of the corn pericarp BG to cellulose suggests the existence of strong interactions between BG and cellulose in corn pericarp.

The distribution of (1,4)-linked glucopyranosyl segments in corn pericarp BG was examined by fragmentation analyses with lichenase which splits (1,3)- β -D-glycosidic linkages in BG (Table 4). After lichenase treatment, cello-octomer and shorter oligomers were detected in the corn pericarp BG. The ratio of cellotriosyl/cellotetraosyl units for corn pericarp BG was 2.5, which was slightly higher than that for barley (2.2). Previously, the same ratios in the native cereal β -glucan structures were reported to be within the range of 1.5-2.3 for oat, 1.8-3.5 for barley, 1.9-3.8 for rye and 3.0-4.5 for wheat (Lazaridou, & Biliaderis, 2007). Ebringerová *et al.* (2005) described in their review as ‘In comparison to the water-extractable β -glucan-rich fractions, the

alkali-extractable ones were characterized by high ratios of cellotriosyl/cellotetraosyl units and large amounts of long, contiguously linked (1→4)-linkage segments. Such polymers exhibit a tendency for interchain aggregation through strong hydrogen bonding along the cellulose-like regions and hence lower solubility'. The present results might fit their descriptions and be suffice to show that the affinity of BG for cellulose was the basis for its isolation.

3. Conclusion

The effectiveness of the combination of anion-exchange and cellulose affinity chromatographic techniques for the isolation of BG from CPHs was established for the first time. By using the present system, the BG present at 6.6% in CPHs was purified to 84.7%. The methylation and fragmentation analyses showed that the BG isolated from the CPHs has (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segment ratios of 2.60 and 2.5, respectively. The chemical structure of the BG was also confirmed by ¹³C-NMR spectroscopic analysis.

Reference

- Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., & Ahmed, Z. (2009). Extraction and characterization of beta-D-glucan from oat for industrial utilization. *International Journal of Biological Macromolecules*, 46(3), 304-309.
- Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., & Din, A. (2007). Physicochemical and functional properties of barley beta-glucan as affected by different extraction procedures. *International Journal of Food Science and Technology*, 44(1), 181-187.
- Beer, M. U., Arrigoin, E., & Amadò, R. (1996). Extraction of oat gum from oat bran: Effects of process on yield, molecular weight distribution, viscosity and (1→3)(1→4)-β-D-glucan content of the gum. *Cereal Chemistry*, 73(1), 58-62.
- Bhatty, R. S. (1993). Extraction and enrichment of (1→3),(1→4)-β-D-glucan from barley and oat brans. *Cereal Chemistry*, 70(1), 73-77.
- Bock, K., Duus, J. O., Norman, B., & Pedersen, S. (1991). Assignment of structures to oligosaccharides produced by enzymic degradation of a beta-D-glucan from barley by ¹H- and ¹³C-n.m.r. spectroscopy. *Carbohydrate Research*, 211(2), 219-233.
- Braaten, J. T., Wood, P. J., Scott, F. W., Wolynetz, M. S., Lowe, M. K., Bradleywhite, P., & Collins, M. W. (1994). Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition*, 48(7), 465-474.
- Brennan, C. S., & Cleary, L. J. (2005). The potential use of cereal

- 251 (1→3,1→4)-β-D-glucans as functional food ingredients. *Journal of Cereal*
252 *Science*, 42(1), 1-13.
- 253 Burkus, Z., & Temelli, F. (1998). Effect of extraction conditions on yield, composition,
254 and viscosity stability of barley β-glucan gum. *Cereal Chemistry*, 75(6),
255 805-809.
- 256 Cui, W., Wood, P. J., Blackwell, B., & Nikiforuk, J. (2000). Physicochemical properties
257 and structural characterization by two-dimensional NMR spectroscopy of
258 wheat beta-D-glucan - comparison with other cereal beta-D-glucans.
259 *Carbohydrate Polymers*, 41(3), 249-258.
- 260 Daou, C., & Zhang, H., (2012). Oat beta-glucan: its role in health promotion and
261 prevention of diseases. *Comprehensive Reviews in Food Science and Food*
262 *Safety*, 11(4), 355-365.
- 263 Ebringerová, A., Hromádková, Z., & Heinze, T. (2005). Hemicellulose. In T. Heinze
264 (Ed.). *Polysaccharides 1: Structure, Characterization and Use* (Vol. 186, pp.
265 1-67). Berlin: Springer-Verlag Berlin.
- 266 Gruppen, H., Hamer, R. J., & Voragen, A. G. J. (1992). Water-unextractable cell wall
267 material from wheat flour. 2. Fractionation of alkali-extracted polymers and
268 comparison with water-extractable arabinoxylans. *Journal of Cereal Science*
269 16(1), 53-67.
- 270 Hakamori, S. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed
271 by methylsulfinyl carbanion in dimethyl sulfoxide. *The Journal of*
272 *Biochemistry*, 55(2), 205-208.

- 273 Izydorczyk, M. S., & Biliaderis, G. C. (1995). Arabinoxylans: advances in structure and
274 physicochemical properties. *Carbohydrate Polymers*, 28(1), 33-48.
- 275 Izydorczyk, M. S., Biliaderis, G. C., Macri, L. J., & MacGregor, A. W. (1997).
276 Fractionation of oat (1→3),(1→4)-β-D-glucans and characterization of the
277 fractions. *Journal of Cereal Science*, 27(3), 321-325.
- 278 Izydorczyk, M. S., & MacGregor, A. W. (2000). Evidence of intermolecular
279 interactions of beta-glucans and arabinoxylans. *Carbohydrate Polymers*, 41(4),
280 417-420.
- 281 Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal beta-glucan
282 functionality: Physical properties, technological applications and physiological
283 effects. *Journal of Cereal Science*, 46(2), 101-118.
- 284 Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). A
285 comparative study on structure-function relations of mixed-linkage (1→3),
286 (1→4) linear beta-D-glucans. *Food Hydrocolloids*, 18(5), 837-855.
- 287 Roubroeks, J. P., Andersson, R., & Aman, P. (2000). Structural features of (1→3),
288 (1→4)-β-D-glucan and arabinoxylan fractions isolated from rye bran.
289 *Carbohydrate Polymer*, 42(1), 3-11.
- 290 Wood, P. J., Weisz, J., Fedec, P., & Burrows, V. D. (1989). Large-scale preparation and
291 properties of oat fractions enriched in (1→3)(1→4)-beta-D-glucan. *Cereal*
292 *Chemistry*, 66(2), 97-103.
- 293 Saeman, J. F., Bubl, J. L., & Harris, E. E. (1945). Quantitative saccharification of wood
294 and cellulose. *Industrial and Engineering Chemistry, Analytical Edition*, 17(1),

- 295 35-37.
- 296 Shukla, R., & Cheryan, M. (2001). Zein: the industrial protein from corn. *Industrial*
297 *Crops and Products*, 13(3), 171-192.
- 298 Yadav, M. P., Johnston, D. B., & Hicks, K. B. (2007). Structural characterization of
299 corn fiber gums from coarse and fine fiber and a study of their emulsifying
300 properties. *Journal of Agricultural and Food Chemistry*, 55(15), 6366-6371.
- 301 Yadav, M. P., Parris, N., Johnston, D. B., & Hicks, K. B. (2008). Fractionation,
302 characterization, and study of the emulsifying properties of corn fiber gum.
303 *Journal of Agricultural and Food Chemistry*, 56(11), 4181-4187.
- 304 Yoshida, T., Tsubaki, S., Teramoto, Y., & Azuma, J. (2010). Optimization of
305 microwave-assisted extraction of carbohydrates from industrial waste of corn
306 starch production using response surface methodology. *Bioresource*
307 *Technology*, 101(20), 7820-7826.
- 308 Yoshida, T., Sakamoto M., & Azuma, J. (2012). Extraction of Hemicelluloses from
309 Corn Pericarp by the NaOH-Urea Solvent System. *Procedia Chemistry*, 4(1),
310 294-300.
- 311 Yoshida, T., Dwianto, W., Honda, Y., Uyama, H., & Azuma, J. (2014). Removal of
312 arabinose substituents from corn pericarp arabinoxylan. *Wood Research*
313 *Journal*. 4(1), 46-50.
- 314
- 315

Figure captions

Fig. 1. Anion exchange chromatographic (A) and affinity chromatographic (B) profiles. (A) The neutral and acidic fractions shown as separate bars at the top of the figure were pooled. (B) Neutral fraction was applied to the cellulose column. The three fractions eluted with SAB, distilled water and 2% NaOH were recovered.

Fig. 2. ^{13}C -NMR spectrum of materials isolated in 2% NaOH fraction using a cellulose column

Table 1. Yields of the separated fractions and sugar compositions of corn pericarp hemicelluloses (CPHs) and separated fractions.

Sample	Yield ^a (%)	Relative monosaccharide composition (% w/w)					β-Glucan content ^b (%)	Starch content ^b (%)
		Ara	Gal	Glc	Xyl	Man		
CPHs	-	29.2	4.1	26.2	40.5	tr ^c	6.6	1.4
<i>Anion exchange chromatography</i>								
Neutral fraction	10.1 ± 0.4	1.0	2.0	80.3	10.1	6.7	28.4	5.0
Acidic fraction	42.8 ± 4.8	35.6	8.2	3.5	50.9	1.9	0.6	0.6
<i>Affinity chromatography on cellulose column</i>								
SAB fraction	7.1 ± 3.2	23.4	7.6	29.5	35.8	3.7	1.3	4.3
Water fraction	1.0 ± 0.7	14.3	7.1	50.6	23.4	4.6	1.4	5.7
2% NaOH fraction	2.6 ± 0.5	1.3	0.9	91.5	6.3	-	84.7 ^d	3.5

^aValues are expressed as a percentage on the basis of the raw material corn pericarp hemicelluloses. Values are expressed as mean ± SD ($n = 3$).

^bValues represent the average of duplication.

^ctr represents trace.

^dValue is expressed as a percentage on the basis of the hot water (100°C)-soluble materials.

333 Table 2. Chemical shifts (ppm) of the ^{13}C responses of the glucose residues of β -glucan isolated
334 in the NaOH fraction in D_2O

Linkage type	Chemical shift (ppm)					
	C1	C2	C3	C4	C5	C6
$\rightarrow 4$)- β -Glc p (1 \rightarrow 3)	105.41	76.08	81.29	76.92	77.68	62.81
$\rightarrow 3$)- β -Glc p (1 \rightarrow 4)	105.22	75.84	86.60	70.81	78.45	63.41
335 $\rightarrow 4$)- β -Glc p (1 \rightarrow 4)	105.22	76.08	81.29	76.92	77.68	62.81

336 Table 3. Methylation analysis of the materials isolated in the 2% NaOH fraction using a
337 cellulose column and of barley β -glucan.

Methylation position	Linkage type	Molar ratio (%)	
		NaOH fraction	Barley β -glucan
2,3,6-Me ₃ -Glc _p	1,4-	71.8	69.4
2,4,6-Me ₃ -Glc _p	1,3-	27.6	29.3
2,3,4,6-Me ₄ -Glc _p	Terminal	0.6	1.0
338 3,6-Me ₂ -Glc _p	1,2,4-	-	0.3

339 Values are expressed as a relative percentage of the total partially methylated glucose residues.

340

Table 4. Fragmentation analysis with lichenase of the materials isolated in the 2% NaOH fraction after affinity chromatography on a cellulose column and of barley β -glucan.

Oligmer	DP	Molar ratio (%)	
		NaOH fraction	Barley
cellobiose	2	0.3	1.3
cello-trimer	3	67.5	63.8
cello-tetramer	4	26.7	28.7
cello-pentamer	5	3.4	4.4
cello-hexamer	6	1.7	1.1
cello-heptamer	7	0.2	0.4
cello-octamer	8	0.1	0.3
cello-trimer + cello-tetramer	3 + 4	94.2	92.5
cello-trimer/cello-tetramer	3/4	2.5	2.2

DP represents degree of polymerization.

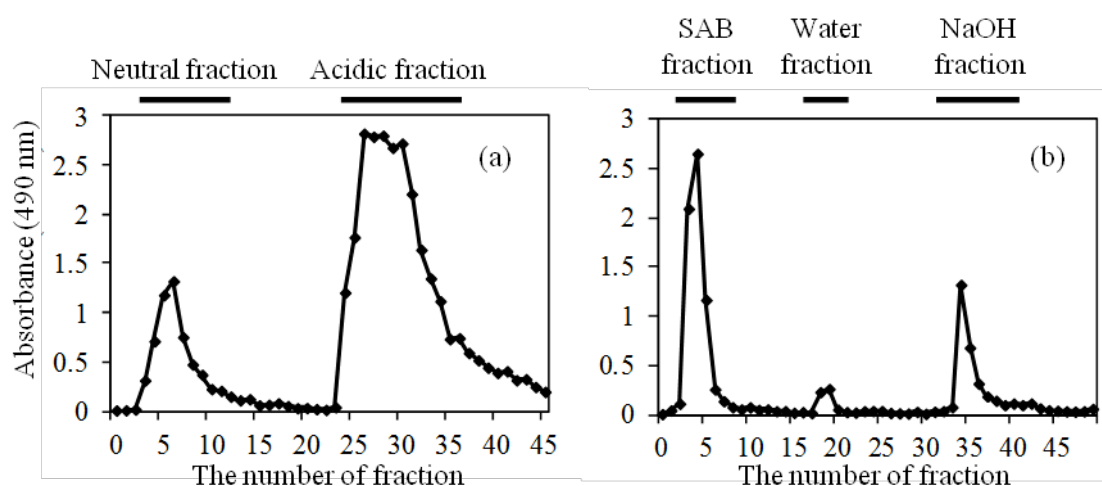
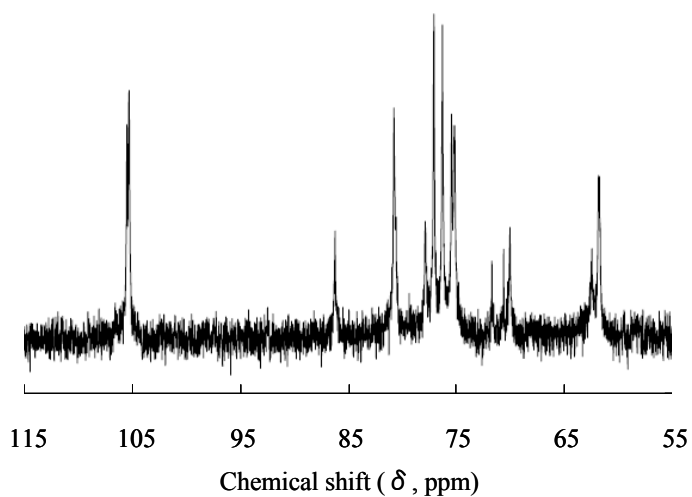


Fig. 1. Anion exchange chromatographic (A) and affinity chromatographic (B) profiles. (A) The neutral and acidic fractions shown as separate bars at the top of the figure were pooled. Fractions 5-15 and 25-36 separated by anion exchange chromatography were designated as neutral and acidic fractions, respectively. The eluent for the anion exchange chromatography was changed at fraction 21 from 5 mM sodium phosphate buffer (pH 6.8) to the same buffer containing 1.2 M NaCl. (B) The neutral fraction was applied to the cellulose column. Three fractions eluted with SAB, distilled water and 2% NaOH were recovered. Fractions 4-11, 19-21 and 35-44 separated by affinity chromatography on a cellulose column were designated as SAB, water and NaOH fractions, respectively. The eluent for the affinity chromatography was changed at fraction 17 from SAB to water, and at fraction 33 to 2% NaOH. The volume of each fraction was 3 mL.



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360 Fig. 2. ^{13}C -NMR spectrum of materials isolated in 2% NaOH fraction using a cellulose column